

Establishment of Compartments
in the Developing Leg Imaginal Discs
of *Drosophila melanogaster*

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Establishment of Compartments in the Developing Leg Imaginal Discs of *Drosophila melanogaster**

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Summary. By X-irradiation of M/M^+ embryos and larvae to induce mitotic recombination, clones of M^+/M^+ genotype were obtained (Fig. 1). Since such cells grow faster than the surrounding M/M^+ -cells they can fill large areas within the compartments of an imaginal disc.

The present studies concentrated mainly on the three leg discs. Clones were induced by doses of 1000 r at ages ranging from 3 ± 0.5 h after oviposition to 144 h.

All clones induced later than the blastoderm stage were absolutely restricted to either the anterior or the posterior compartment of a disc. The border between the anterior and posterior compartment runs as a straight line along the entire leg and at the distal end separates the two claws (Figs. 5, 6, 7). A further subdivision of the anterior compartment is indicated by clones initiated in the second larval instar (Fig. 11). A parallel subdivision could not be detected in the posterior compartment. Irradiation in the early third instar led to clones which were restricted to single longitudinal bristle rows and leg segments. But no clear-cut compartment borders could be found; in particular a proximo-distal separation appears to be absent.

Among the 318 clones induced at the blastoderm stage eleven extended from the wing into the second leg (Fig. 8), or from the haltere into the third leg.

With the exception of 3 clones that apparently occupied the anterior as well as the posterior compartment of a wing or a leg, all clones remained confined to either the anterior or the posterior compartment.

Frequently clones overlapped left and right forelegs (Fig. 9). Intersegmental overlaps were not observed.

The results show that the earliest compartment borders appear in all thoracic discs. This suggests that compartmentalization is a fundamental process common to all discs.

Key words: Imaginal discs — Compartments — Clonal analysis — Embryonic determination.

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Numerous experiments suggest that determination in imaginal discs of *Drosophila* occurs progressively and begins early in the embryo (reviews by Nöthiger, 1972; Gehring, 1972). Cells from the blastoderm stage are determined at least with respect to anterior or posterior adult structures (Hadorn et al., 1968; Chan and Gehring, 1971; Illmensee and Mahowald, 1974). Treatment of wildtype blastoderms with ether or heat shock (Henke and Maas, 1946; Gloor, 1947) resulted in phenocopies of the *tetraptera* or *bithorax* mutant (Bridges and Morgan, 1923; Astauroff, 1929). Both these mutations transform the metathorax into a second mesothorax. These findings suggest that a segment-specific determination already takes place at the blastoderm stage. Recent cell lineage studies using mitotic recombination during embryogenesis support the existence of segment-specific determination (Wieschaus and Gehring, 1976a). Clones induced at the blastoderm stage could extend over the dorsal and ventral disc of a larval segment (wing and 2nd leg) but no clones were ever found crossing segmental borders. Similar segmental restrictions during blastoderm formation are also reported to occur in the abdomen of *Oncopeltus* (Lawrence, 1971, 1973, 1975).

The imaginal disc cells of a third instar larva are clearly determined with a much higher degree of specificity than those of the early embryo. By cutting discs into small fragments and implanting these fragments separately into larval hosts each region of the disc could be assigned a specific set of adult structures (Hadorn et al., 1949; Ursprung, 1959; Hadorn and Buck, 1962; Schubiger, 1968; Bryant, 1975). Developmental restrictions that occur progressively during the larval period should be detectable by X-ray-induced mitotic recombination (Stern, 1936; Becker, 1957). With this method the progeny of a single cell can be marked at a chosen time of development. If the marked cell was already determined at the time of irradiation its clonal descendents will be restricted to forming particular adult structures. Such restrictions could in fact be found in several discs at different times of larval life (Becker, 1957; Bryant and Schneiderman, 1969; Bryant, 1970; Garcia-Bellido, 1968; Garcia-Bellido and Merriam, 1971; Postlethwait and Schneiderman, 1971). However, the clone size decreases, the later during larval development mitotic recombination is induced. It can therefore be argued that the restrictions observed in normal cell lineage studies were only an artifact due to limited proliferation. Recently, however, Morata and Ripoll (1975) found a method for producing larger clones. It is based on the observation that *Minute* heterozygotes grow at a slower rate than wild type larvae. A suitably marked M^+/M^+ cell induced in a *Minute* heterozygous imaginal disc will divide faster than the surrounding cells. The resultant M^+/M^+ clone therefore occupies a relatively much larger region in the adult surface. Depending on the time of induction of the M^+/M^+ clone, it will not cross certain demarcation lines, but will run along them for great distances in spite of its large size. These findings have led to the hypothesis that a disc becomes progressively subdivided into defined regions, called compartments (Garcia-Bellido et al., 1973, 1976). Each compartment derives from a group of founder cells (Garcia-Bellido et al., 1973; Lawrence, 1973). Once a decision to form a particular compartment is taken, it is maintained in the clonal descendents of the founder cells. Thus each compartment is a polyclone

(Crick and Lawrence, 1975) of equipotent cells that can replace each other. If one cell of a polyclone divides faster than the others it overgrows them and will practically fill an entire compartment. But the resulting adult structure is of normal size and shape.

In the wing disc the first compartments are of anterior and posterior quality and already seem to exist in the embryo. Clones crossing the anterior-posterior compartment border were never observed. The basic importance of this border was also indicated by the finding that certain morphogenetic mutants exclusively affect one or an other of these compartments. For example, the mutant *engrailed* (*en*) converts the posterior compartment of the wing into a second anterior one (Garcia-Bellido and Santamaria, 1972; Garcia-Bellido, 1975; Morata and Lawrence, 1975; Lawrence and Morata, 1976).

In this paper we will show that compartmentalization occurs in the leg discs as well and thus appears to be a general developmental property of all the thoracic discs.

Material and Methods

Genetical Markers

In *Drosophila melanogaster* large regions of the adult legs lack bristles. Therefore we used marker genes which affect both bristles and trichomes for the clonal analysis. We have chosen the following genetic system to induce mitotic recombination: *y; Dp(1;3) sc¹⁴, y⁺ M(3)i⁵⁵/mwh jv* from the cross non-*Minute* females $\times$ *Minute* males. Mitotic exchange proximal to *M(3)i⁵⁵* in the left arm of the third chromosome results in *y; mwh jv* spots on the adult surface (Fig. 1). For a description of the mutants see Lindsley and Grell (1968). *Yellow* (*y*) bristles are excellent markers on the legs except for the tarsal segments where wildtype bristles are also lightly pigmented. *Javelin* (*jv*) has a clear effect only on large bristles and was not very useful. *Multiple wing hairs* (*mwh*) changes the trichome pattern in all body regions, mainly by increasing the number of hairs and by disturbing their orientation (Peyer and Hadorn, 1965). It is therefore easily detectable. *M(3)i⁵⁵*, a strong *Minute*, maps at 3-27.1 (Garcia-Bellido et al., 1973).

Irradiation

Mitotic recombination was induced by X-irradiation with a Picker machine (200 kV, 12 mA, 1.0 mm Al-filter, focus distance 29.5 cm, 214 r/min). Eggs from 24 h collections were allowed to develop on standard food at $25 \pm 0.5^\circ \text{C}$ until the oldest individuals reached the age of 24, 48, 72, 96, 120 or 144 h respectively, and were then irradiated. The total dose applied was 1,000 r in all irradiations except for those made at 24 h where 500 r were used to ensure reasonable survival. Besides these mainly larval stages, clones were also induced in blastoderms at 3 ± 0.5 h after oviposition. A first series of irradiations was carried out with a Philips MG 150 X-ray machine (100 kV, 10 mA, 1.7 mm Al-filter, 1 mm plexiglass, focus distance 14 cm, 653 r/min); a second one with a Siemens Dermopan 2 (50 kV, 25 mA, 0.3 mm Al-filter, 1 mm plexiglass, focus distance 30 cm, 213 r/min). To ensure that the treated embryos were really blastoderms we dechorionated and examined some egg collections after irradiation. In bottles with embryos that had not been examined, *Minute* flies which hatched earlier than ten days after oviposition were discarded.

Preparation and Scoring

We fixed the hatched *M/M⁺* flies for several days in a solution of glycerine and alcohol. The thoraces were then isolated and opened by a clean ventral cut between the legs with a microscalpel.

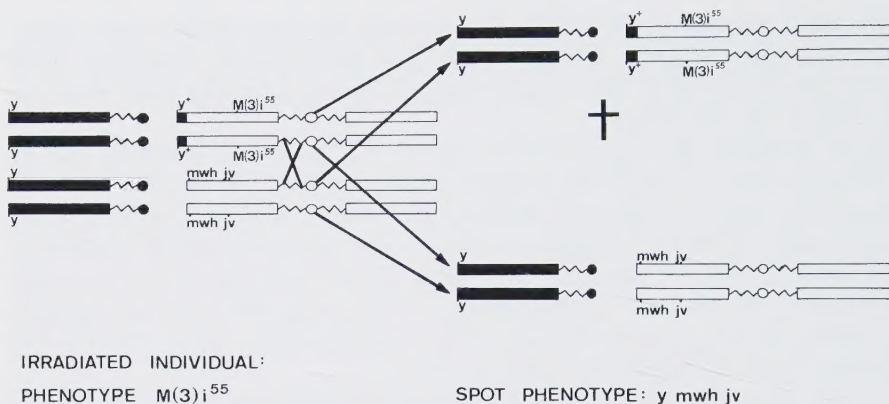


Fig. 1. Schematic representation of mitotic recombination leading to the mosaic flies examined. The homozygous M/M constitution is cell-lethal, preventing the formation of twin spots. Black: first chromosome, white: third chromosome

The thoraces were heated in 10% KOH for 10 min at 80° C and washed twice in distilled water at the same temperature. They were then transferred to 100% alcohol and mounted in Euparal between coverslips for microscopical analysis.

Spots were outlined on standard drawings. The standard bristle pattern (Fig. 6) is based on an analysis of ten flies. For orientation of the legs and for bristle arrangement previous publications were very useful (Hannah-Alava, 1958; Hollingsworth, 1964; Bryant and Schneiderman, 1969). In the nomenclature of the structures on the ventral thorax we followed Zalokar (1947).

Statistics

To determine whether apparently overlapping clones were in reality due to two separate inductions, the binomial distribution was used to estimate the probability of independent inductions. For anterior-posterior and intersegmental overlaps the probability of two simultaneous clones was calculated for all pairs of discs (or compartments), and the sum of these individual probabilities was used to estimate the frequency of any such overlap in the total number of half thoraxes examined.

Minute Development and Cell Counts

The delay of $M(3)i^{55}$ during development was measured as follows: For all stages we used one-hour egg collections from the cross $y; \text{mwh } jv \times y; Dp(1:3) \text{ } sc^{14}, y^+ M(3)i^{55}/TM1$. Its progeny, yellow non-*Minute* flies ($y; TM1/\text{mwh } jv$) and *Minutes* ($y; Dp(1:3) \text{ } sc^{14}, y^+ M(3)i^{55}/\text{mwh } jv$), are easily distinguishable already in the first larval instar by the different colour of their mouthhooks. 20 to 25 h after oviposition newly hatched larvae were isolated in intervals of 30 min and counted according to their genotype (Fig. 2a). To examine the delay of larval moults, 100 or 200 eggs from 1 h collections were transferred into separate vials. Between 40 and 88 h after egg laying we stopped development in one vial every hour by freezing. The larvae were washed out and classified according to their genotype and larval stage (Fig. 2b, c). The mouthhooks in the three larval stages are of characteristic shape and size (Bodenstein, 1965). From each vial we measured the lengths of ten larvae of each genotype and stage existing at the given time. The delay in puparium formation and adult hatching was examined by hourly collections of pupae or adults, respectively (Fig. 2d, e).

Wing discs were dissected from 50 h old larvae and from freshly hatched third instar larvae of both genotypes. Leg discs at this stage, especially from *Minutes*, are too small to be isolated cleanly. Wing discs are attached to a tracheal branch and are therefore easy to isolate even if they are very small.

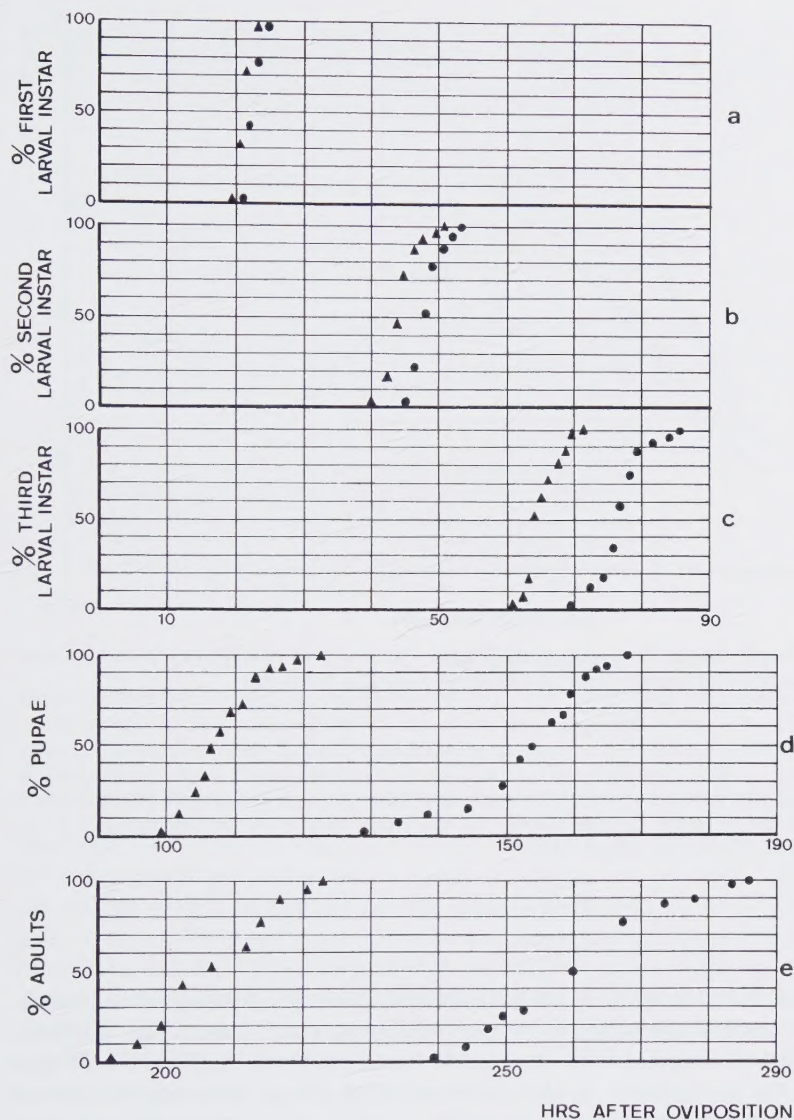


Fig. 2. Cumulative delay of $M(3)i^{55}$ -individuals at different developmental stages in comparison to non-*Minutes* from the same parents (at 25° C) (a-e see Material and Methods). ▲ $y; TM\ l/mwh\ jv$; ● $y; Dp(1;3)sc^{14}, y^+ M(3)i^{55}/mwh\ jv$

The discs were treated on a slide with a solution of 0.1% trypsin in Ringer's for 15 min. Then the action of the trypsin was stopped by adding a drop of fetal bovine serum. The peripodial membrane was often not fully digested and had to be disrupted slightly with tungsten needles. We squashed the discs under a coverslip by adsorbing the liquid with blotting paper under microscopical observation until the cells were spread as a monolayer. The cells were counted on enlarged photographs. Wing and leg discs from larvae ready to pupate were treated in the same way, but only about 1/7 of a disc was photographed. The whole outline of the squashed disc and

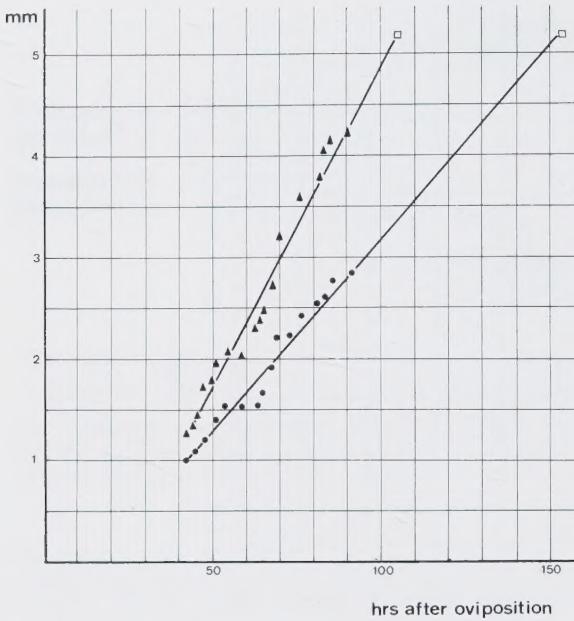


Fig. 3. Larval length during development of *Minutes* (●) and non-*Minutes* (▲) from the same parents. □ Average larval length of both genotypes at puparium formation

the photographed part were drawn with a camera lucida. These areas were measured planimetrically relative to each other. Hence the cell number of the whole disc could be calculated. 6 to 10 discs were examined per genotype and stage.

Results

Heterozygous *Minutes* show a mutant-specific delay in development, mostly due to slower growth during the larval period (Dunn and Coyne, 1937; Brehme, 1939; Morata and Ripoll, 1975). One of the major problems in using the *Minute* technique is the correlation of the developmental age of the retarded larvae with that of a wildtype standard. In order to make this comparison we have measured the developmental rate of the *M(3)i⁵⁵* compared to their non-*Minute* siblings (Fig. 2). Very little delay is observed during embryogenesis or the pupal period. The retardation of development occurs mostly during larval life with an increased variance at older stages. The increase in larval length was found to be more or less linear, but different for *Minutes* and non-*Minutes* (Fig. 3).

Freshly hatched third instar larvae from both genotypes were found to be of very different size. Cell counts were made on wing discs at 50 h of development and at the beginning and at the end of the third larval instar. The average cell number of wing discs removed from larvae 50 h old was 247 ± 8 for non-*Minutes* and 87 ± 3 for *Minutes*. The cell number of wing discs from freshly hatched third instar *M* larvae was less than half (458 ± 23) of

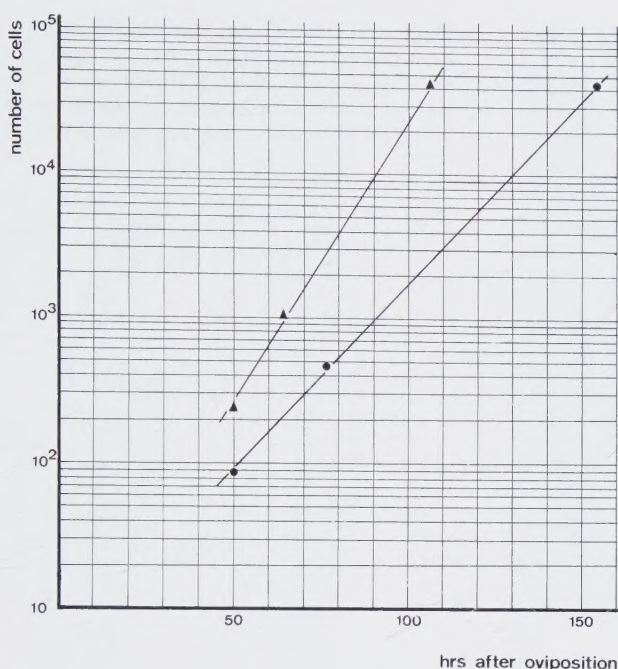


Fig. 4. Extrapolated growth curves of M^+/M^+ ($\blacktriangle$) and M/M^+ ($\bullet$) wing imaginal discs based on cell counts at 50 h of development and at the beginning and at the end of the third larval instar. Previous work using mitotic recombination supports an exponential growth of both *Minute* and non-*Minute* discs (Bryant, 1970; Garcia-Bellido and Merriam, 1971; Morata and Ripoll, 1975)

the wildtype number (1024 ± 32). Shortly before puparium formation, however, the cell number per wing disc is equal in both genotypes (M^+ : $43,400 \pm 1,170$, M : $43,290 \pm 900$). In mature foreleg discs we counted about 21,000 cells. The cell counts of the wing discs have been summarized in Figure 4. The doubling time is 8 h for M^+/M^+ cells and 12 h for M/M^+ cells. If determination during development is dependent on the cell number in a given disc and not on its absolute age, then Figure 4 can be used to correlate development of M/M^+ and M^+/M^+ . For example 120 h of *Minute* development would correspond to 84 h of non-*Minutes*. However a *Minute* larva entering the third instar has only half the number of wing disc cells as its non-*Minute* sibs. This is true in spite of the fact that the early third instar *Minute* larva is 12 h older. Thus relative to the physiological age of the larvae measured in moults, most of the delay in growth of imaginal discs occurs before the 3rd instar, since the numbers of cells in *Minute* and non-*Minute* wing discs are the same at the time of pupation.

The Early Compartments

M^+/M^+ clones induced in the leg primordia of late M/M^+ embryos or young larvae occupy very large areas in the adult. However, already at this stage



Fig. 5. a An anterior-posterior compartment border in the femur of the midleg. **b** Claws of different genotype. Anterior compartment marked with *y; mwh jv*, posterior compartment with dark coloured bristles and claws (*M(3)i⁵⁵*)

the clones are restricted to either an anterior or a smaller posterior region in each disc. In defining the compartment border we have relied on certain large landmark bristles and on the vertical bristle rows since the exact bristle pattern varies from leg to leg. This boundary runs as a straight line from the thorax along the length of each leg and separates, at the most distal part, the two claws (Fig. 5a, b). In fact no clones were ever found to include both claws even when the irradiation was made at 3 h (Fig. 5b). Figure 6 demonstrates on a representative bristle pattern the exact outline of these early compartments in the three legs.

The anterior-posterior demarcation line divides the male foreleg basitarsus into two sections that are identical with the regions A and B described by Tokunaga (1962) who found them to have independent cell lineages.

It is remarkable that the compartment boundaries in the thorax (Fig. 7) do not always follow anatomical sutures but often run through areas covered

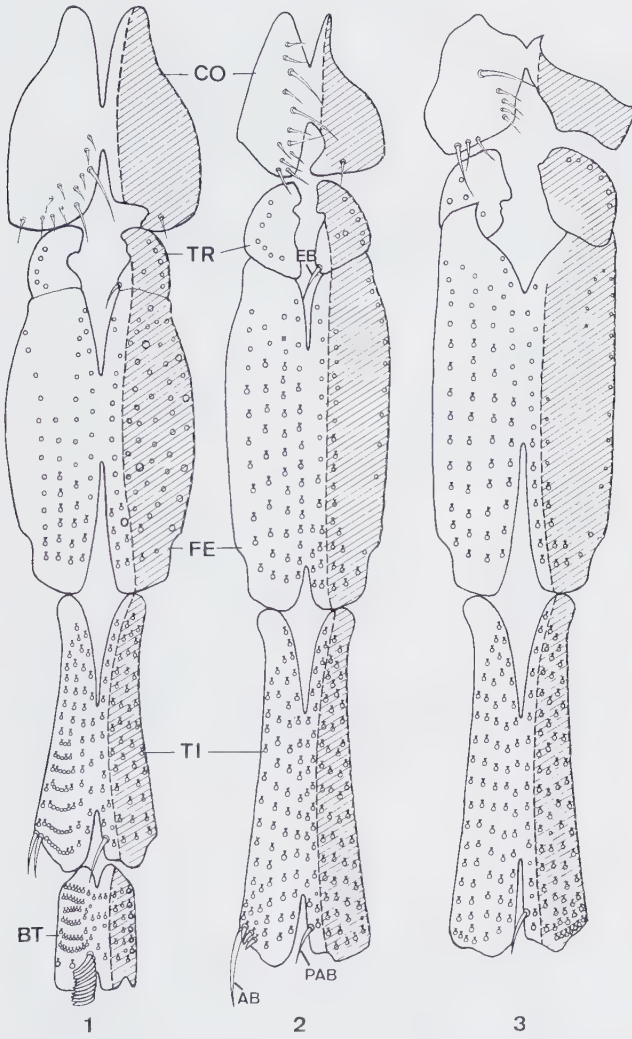


Fig. 6. The anterior (blank) and posterior (hatched) compartments in the three legs (1-3). Broken lines: anterior-posterior compartment borders. CO coxa, TR trochanter, FE femur, TI tibia, BT basitarsus, EB edge bristle, PAB preapical bristle, AB apical bristle

homogeneously with trichomes. Both the *propleura* and *sternum I* are part of the anterior foreleg compartment. The small *epimerum I* which is not separated from the *sternopleura* by a suture belongs to the posterior foreleg. The large *sternopleura* plus a triangle of the *hypopleura* are components of the anterior midleg compartment. The posterior midleg occupies a precisely defined middle part of the *hypopleura*. The rest of the *hypopleura* and the *episternum III* were found to be descendants of the anterior hindleg. The small *epimerum III* belongs to the posterior hindleg compartment. Thus the *hypopleura* is built up from three different compartments of two different discs. Zalokar (1943) found a similar participation of the leg discs in forming the ventral thorax. However,

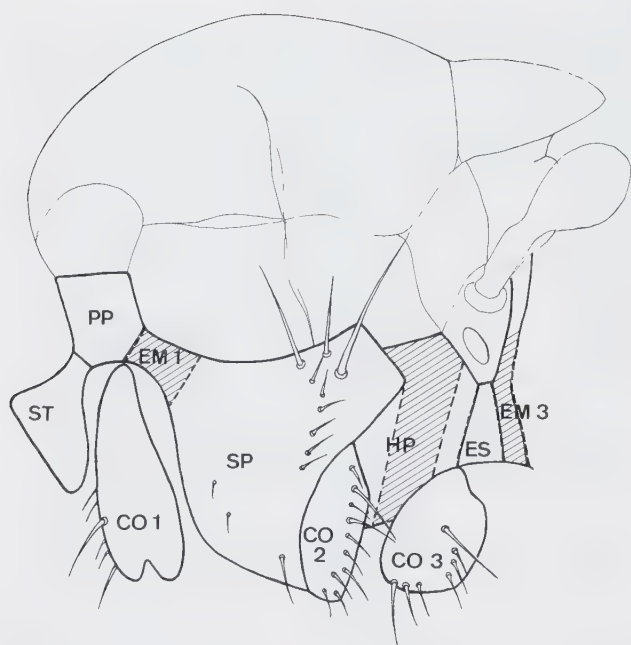


Fig. 7. The components of the anterior (blank) and posterior (hatched) leg-compartments in the thorax (see text). Broken lines: anterior-posterior compartment borders. The derivatives of the leg discs are surrounded by thick lines, those of the dorsal thoracic discs by fine lines. *PP* propleura. *ST* sternum I, *EM 1* epimerum I, *SP* sternopleura, *HP* hypopleura, *ES* episternum III, *EM 3* epimerum III. *CO 1-3* coxa 1-3

the ablation technique he used in mature larvae did not permit detailed analysis of these contributions.

The few clones induced in embryos (12 ± 12 h after oviposition, Table 2) by irradiation with 500 r never crossed the anterior-posterior demarcation line. However, because early embryonic stages are highly sensitive to X-rays this result tells us little about restriction in early embryos as only the older embryos might have survived the treatment. It was therefore interesting to know whether these first compartments already exist in the blastoderm stage. Blastoderms ($2\frac{1}{2}$ to $3\frac{1}{2}$ h after oviposition) have been reported to be relatively insensitive to X-ray damage and about 50% are expected to survive after irradiation with 1,000 r (Fritz-Niggli, 1955; Wieschaus and Gehring, 1976a). Spots induced by mitotic recombination at this early stage were recently shown to overlap wing and 2nd leg structures (Wieschaus and Gehring, 1976a). In a first series (Table 10) we have examined 446 *M*-flies irradiated at 3 ± 0.5 h (blastoderm), in a second series 958 *M*-flies irradiated at the same time but using different parameters (see p. 11). The total dose in both series was 1,000 r. We found thoracic clones which were extremely large and often even filled an entire compartment.

Frequently in both series either the anterior or the posterior compartment was marked on both forelegs. This left-right overlap is statistically highly significant when induced at the blastoderm stage (p for independent events < 0.0001)

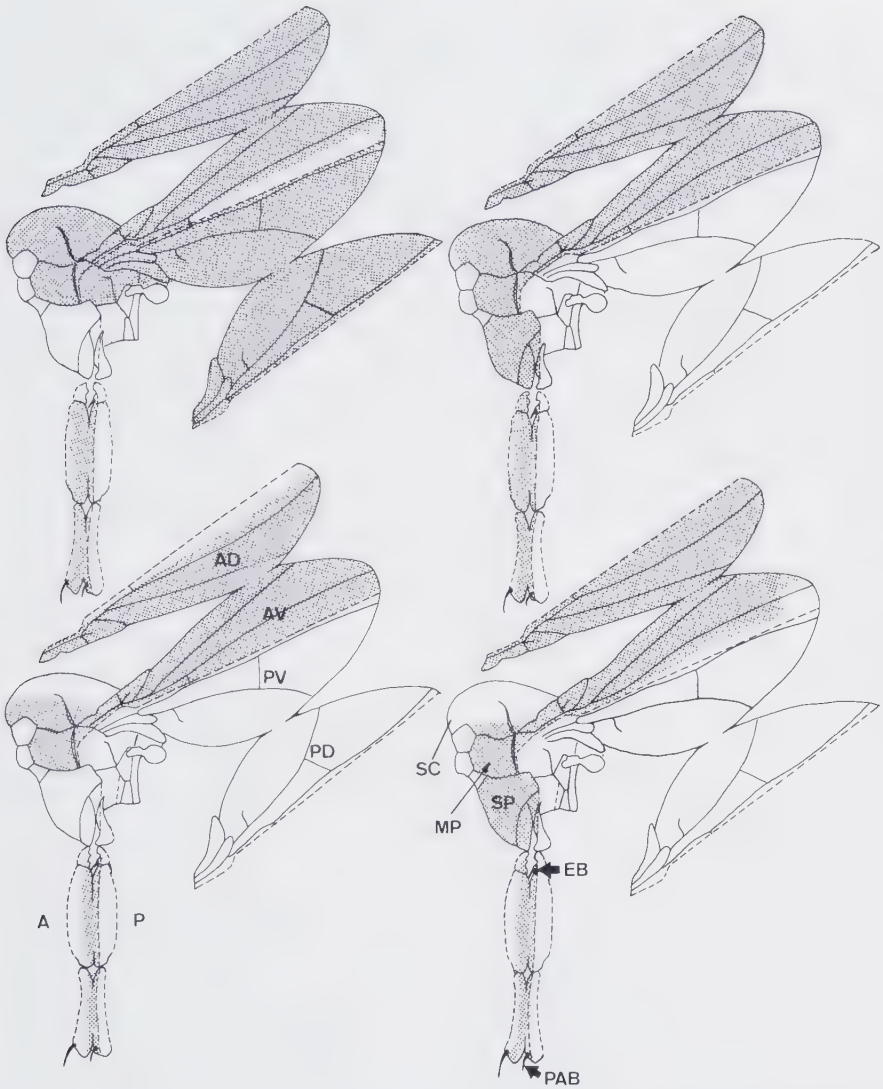


Fig. 8. Overlapping clones of second leg and wing induced at the blastoderm stage. Wing and leg opened at an anterior-posterior compartment border (broken lines). *A* anterior, *P* posterior, *V* ventral, *D* dorsal, *SC* scutum, *MP* mesopleura, *SP* sternopleura, *EB* edge bristle, *PAB* preapical bristle

and was still found after irradiation at 24 to 48 h (3 cases $p=0.02$), but not if irradiated later. Thus, the left-right separation in the foreleg does not occur until the middle of the first instar.

In the first series four clones were detected overlapping the second leg and wing ($p=0.009$). Wieschaus and Gehring (1976b) using gynandromorph mapping (Sturtevant, 1929; Garcia-Bellido and Merriam, 1969; Hotta and Benzer, 1973)

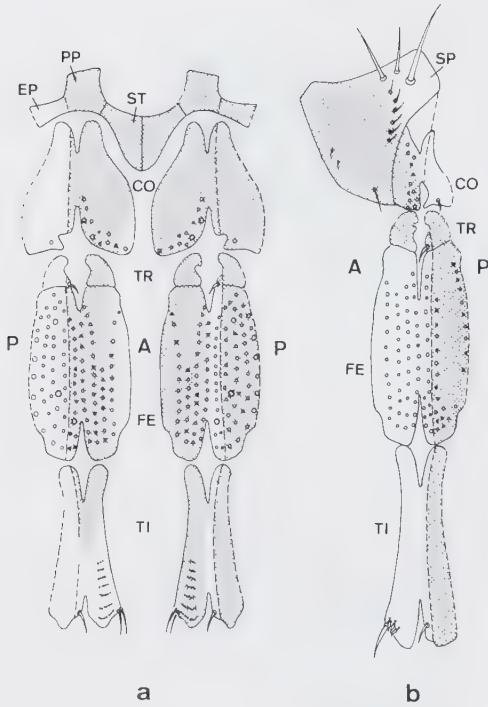


Fig. 9a and b. Anterior-posterior overlaps of clones induced at the blastoderm stage. **a** Anterior-posterior and left-right overlap in forelegs. **b** Anterior-posterior overlap in a midleg. Shaded areas: extent of these clones. Broken lines: anterior-posterior compartment borders. *A* anterior, *P* posterior, *ST* sternum I, *PP* propleura, *EP* epimerum I, *CO* coxa, *TR* trochanter, *FE* femur, *TI* tibia, *SP* sternopleura

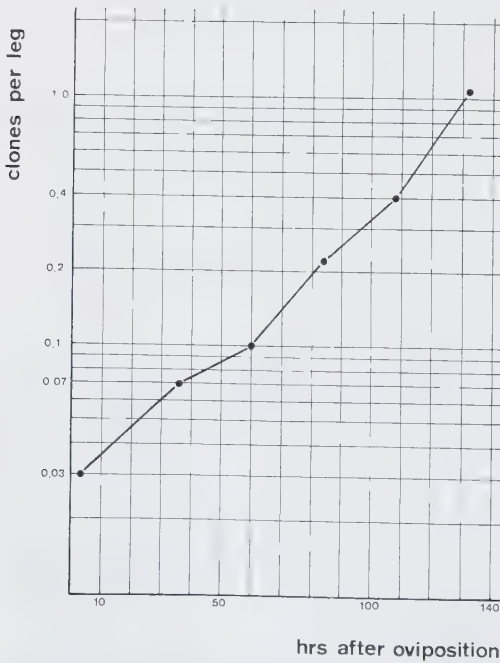


Fig. 10. Semilogarithmic plot of clone frequencies induced with 1,000 r X-rays at different developmental stages

found that in the blastoderm the *edge bristle* (EB) and the *preapical bristle* (PAB) lay closer to the wing base than the bristles of the *sternopleura*, which is in contrast to the situation in the adult. Consistent with these findings, two of our four overlapping clones, in spite of their large size, avoided *sternopleura* and coxa but included wing base and *edge bristle* or *preapical bristle* (Fig. 8). These four overlapping clones occupied the anterior compartments of both the wing and 2nd leg, but in one wing the clone nearly filled the posterior compartment as well.

In the second series two of the four clones found to overlap wing and second leg were restricted to the posterior compartments, the others to the anterior ones. All these four clones were continuous.

We also observed one case in the first series and two cases in the second series where clones marked both 3rd leg and haltere. Two of them were restricted to the anterior, one to the posterior compartments.

In the first series three anterior-posterior overlaps were found: the previously mentioned one in a wing (Fig. 8), one in a foreleg and the third in a midleg (Fig. 9). In these cases, the clones occupied large areas of both anterior and posterior compartments. The continuity of the marked patches and the low probability for two independent inductions ($p=0.02$, calculated for series I) make it very likely that the overlapping regions were true clones. The failure to find such overlaps in the second series will be discussed later.

The expected frequency of double inductions occurring in two adjacent thoracic segments was 7.7 in 2,808 half thoraces (both series). We found eight such cases, and in only one of these two adjacent compartments were marked (posterior wing and anterior hindleg). Thus, blastoderm clones were not found to cross segmental borders, indicating that segmentation has already taken place at this stage.

Clone Frequencies and Later Restrictions

In Table 2 we present the clone frequencies obtained after irradiation at different time intervals, and Figure 10 shows a semilogarithmic plot of these data. In order to detect later compartment borders we superimposed all the outlines of spots induced in a given age interval. Figure 11 shows the results for the femur of the first leg. A demarcation line appears between the bristle rows II and III, dividing the anterior compartment into two longitudinal halves of dorsal and ventral quality (Fig. 11b, c arrows). This line does not seem to be defined as precisely as the anterior-posterior compartment border. Instead its position between the two rows varies in different flies. After irradiation at 84 ± 12 h (60 ± 12 h wildtype time) no clone crossed this border in the foreleg, and in the midleg only 2 clones crossed it, in comparison to 18 clones which touched it. However, even after irradiation at 108 ± 12 h (76 ± 12 h wildtype time) two clones were found to cross this line in the hindleg compared to 26 clones that touched the border but did not overlap. At this later time the border was never crossed in the midleg.

In the smaller posterior compartment the leg segment best suited for observation of an analogous subdivision is the femur. However, the posterior midleg

Table 1. Number of marked compartments in M/M^+ flies irradiated at the blastoderm stage. In parenthesis: number of foreleg clones if left-right overlaps are counted as one clone (see text)

Number of flies examined	Foreleg		Midleg		Hindleg		Wing		Haltere		Total	Frequency of clones	
	ant.	post.	ant.	post.	ant.	post.	ant.	post.	ant.	post.		per leg	per wing
1st series:													
446	21 (15)	12 (8)	22	11	13	5	15	8	5	0	112	0.027	0.025
2nd series:													
958	39 (30)	17 (13)	27	12	40	15	26	16	12	2	206	0.024	0.022

Table 2. M^+/M^+ clone frequencies after irradiation of M/M^+ embryos and larvae at different time intervals

Age of Minutes at irradiation (h after oviposition)	Corresponding age in wildtype standards (Fig. 4)	Number of flies examined	Total number of clones on legs	Clone frequency (clones per leg)	Distribution of clones on the ant./post. leg compartments					
					Foreleg		Midleg		Hindleg	
					ant.	post.	ant.	post.	ant.	post.
3 ± 0.5 h	3 ± 0.5 h	1,404	212	0.03	45	21	49	23	54	20
12 ± 12 h	12 ± 12 h	133	15	0.02 ^a	1	1	4	0	7	2
36 ± 12 h	28 ± 12 h	106	42	0.08	13	6	10	6	6	1
60 ± 12 h	44 ± 12 h	183	109	0.1	20	14	37	8	26	4
84 ± 12 h	60 ± 12 h	261	337	0.22	59	47	112	33	77	9
108 ± 12 h	76 ± 12 h	126	304	0.4	59	38	88	22	81	16
132 ± 12 h	92 ± 12 h	50	325	1.08	53	45	91	26	95	15

^a This low frequency is produced with an irradiation dose of only 500 r, owing to the low survival of embryos irradiated during this interval

and hindleg femora carry only a few bristles, and large areas lack even trichomes. The femur of the foreleg has more markers, but no clear demarcation-line could be found within the posterior compartment. Figure 11b and c show that clones tend to be restricted to bristle rows, but no clear border could be identified. The analysis of the other posterior leg segments provided no better distinction.

At about 80 h (wildtype time) after oviposition a proximal-distal border becomes established in the wing (Garcia-Bellido et al., 1973, 1976). A likely site for such a border in the leg would be a separation of two particular leg segments. However, in spite of the large number of clones induced at 108 ± 12 h (76 ± 12 h wildtype time), relatively few were found in the regions around leg joints. Table 3 gives, for different irradiation intervals, the numbers of clones stopping precisely at a given leg joint and the number of those overlapping the two adjacent leg segments. These data show that clones are not preferentially

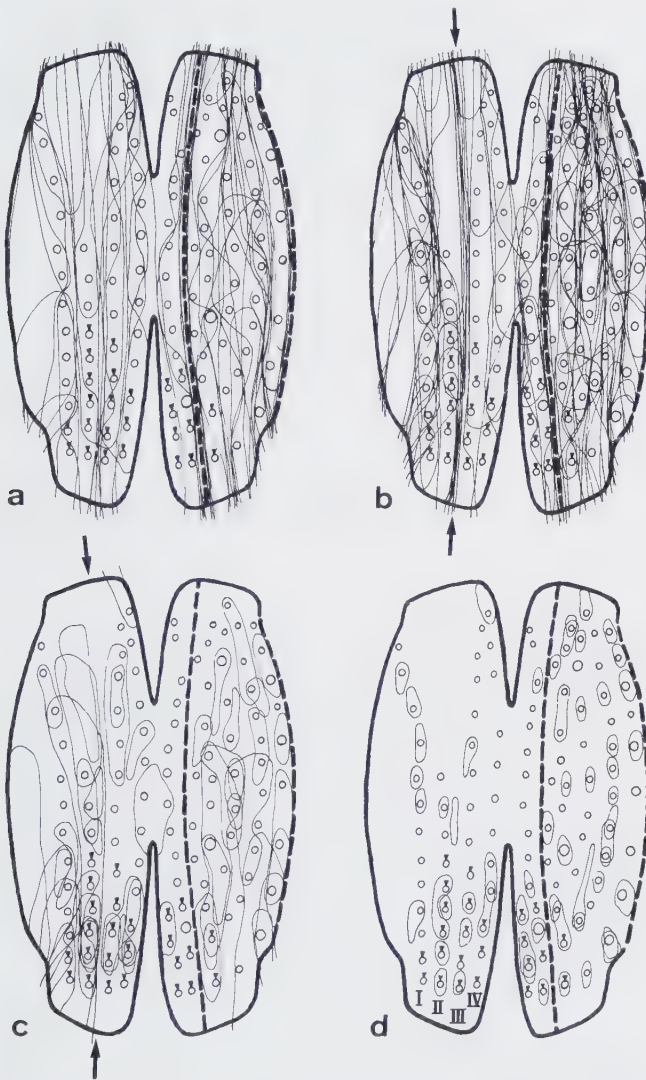


Fig. 11a-d. Superimposed outlines of clones on the foreleg femur induced at different age intervals: **a** 60 ± 12 h, **b** 84 ± 12 h, **c** 108 ± 12 h, **d** 132 ± 12 h. Broken lines = anterior-posterior compartment borders. Arrows point to the dorsal-ventral restriction in the anterior compartment. I, II, III, IV bristle rows in the anterior compartment. Clone outlines drawn over the borders of the femur extend into adjacent leg segments

restricted to a particular joint. Thus, a distal-proximal border analogous to that found in the wing seems to be absent. However, the small clones produced after 120 h (84 h wildtype time) never extended into more than one leg segment but were restricted to single bristle rows. When converted to wildtype time these results agree very well with the findings obtained from normal cell lineage by Bryant and Schneiderman (1969).

Table 3. Number of overlapping (overl.) and stopping (stop.) clones at the different leg joints. Th thorax, Co coxa, Tr trochanter, Fe femur, Ti tibia, Ta tarsus, F forelegs, M midlegs, H hindlegs

Minute-time of irradiation		Th-Co		Co-Tr		Tr-Fe		Fe-Ti		Ti-Ta		Number of legs examined	Total number of clones on these legs
		overl.	stop.	overl.	stop.	overl.	stop.	overl.	stop.	overl.	stop.		
84 ± 12 h	F	6	0	15	2	14	19	19	21	14	1	522	106
	M	13	18	15	4	16	23	24	28	14	3	522	145
	H	8	1	8	7	8	13	17	16	9	2	522	86
108 ± 12 h	F	0	0	2	9	1	2	3	17	5	4	252	97
	M	3	13	2	10	1	8	4	8	6	3	252	110
	H	2	0	2	8	1	8	1	17	1	1	252	97
132 ± 12 h	F	0	0	0	1	0	0	0	1	0	1	100	98
	M	0	0	0	0	0	0	0	0	0	2	100	117
	H	0	0	0	0	0	0	0	1	0	0	100	110

Discussion

Progressive determination in imaginal discs can be visualized most easily by the process of compartmentalization as it occurs in the wing disc (Garcia-Bellido et al., 1973; 1976): The earliest compartment border separates a large anterior from a smaller posterior region. Later in development, probably in the first larval instar, each of these two compartments is subdivided into four parts. The new compartment borders divide dorsal from ventral regions and wing from notum. In the third larval instar the prospective scutum becomes separated from the pleura and the wing is subdivided proximo-distally. Once established, each of these compartment borders is maintained throughout subsequent development and clones are never found to cross them.

In the legs we found clearly defined anterior and posterior compartments very early in embryogenesis as well. The border runs along the legs, separating the two claws at its most distal point. Posterior compartments are smaller than the anterior ones, analogous to the earliest compartments of the wing. At about 50 to 70 h of non-*Minute* development a further restriction could be shown subdividing the anterior compartment into a dorsal and a ventral region. This border probably corresponds to the dorsal-ventral demarcation line in the wing. It does not seem to be as clearly defined as the dorsal-ventral border in the wing or the anterior-posterior border in the leg, however. In the small posterior compartments of the legs no clear division could be detected. We also failed to find a proximo-distal compartment border.

The blastoderm stage must be a special focus of interest in any attempt to reveal the earliest developmental restrictions. This is the first study in which the relationships between different discs have been analyzed in the blastoderm using the *Minute* technique. During blastoderm formation the cleavage nuclei on the egg periphery are separated by cellular membranes and thus come to lie in a particular region of the cortex. Some authors have suggested that disc

determination occurs at this time (Bryant and Schneiderman, 1969; Bryant, 1970; Garcia-Bellido and Merriam, 1971; Postlethwait and Schneiderman, 1971). The recent findings of Wieschaus and Gehring (1976a) that clones induced at the blastoderm stage can extend over both the second leg and wing, however, support the idea of determination being only segment-specific at the blastoderm stage.

Accordingly, we found eight cases of overlaps between wing and second leg, and three cases between haltere and third leg (combining the observations from both series). Although they extended from the dorsal into the ventral disc within a given segment, these clones were, with one exception (Fig. 8), restricted to either the anterior or the posterior compartments. One could regard disc-determination within a segment to be an additional step in compartmentalization, possibly occurring after the segments or anterior and posterior compartments have been established (see p. 27).

Very frequently we observed left-right overlaps in the forelegs. Either the anterior compartments were marked on both sides, or the posterior ones. Such overlap occurs after irradiation of embryos and larvae up to 48 h old (*M*-time). The forelegs marked on both sides were normal and not fused in the manner described by Postlethwait and Schneiderman (1973) due to X-ray damage. Data of gynandromorph mapping indicate that left and right foreleg primordia lie far apart in the blastoderm (Hotta and Benzer, 1973; Wieschaus and Gehring, 1976b). The question then is how this overlap over great distances can occur. One possible explanation is the fusion of the right and the left foreleg primordia during gastrulation after the ventral invagination. If the determination for left and right had not previously taken place, a rapidly growing clone induced on one side could easily invade the other side.

We could not find any intersegmental overlaps, although the distance between midleg and hindleg in the blastoderm is about the same as between midleg and wing (Wieschaus and Gehring, 1976b). If intersegmental overlaps occur, their frequency must be so low that they are not distinguishable from those cases where two clones were induced independently in adjacent discs.

Some of the reservations necessary for interpreting clones induced at 3 ± 0.5 h of development have been discussed by Wieschaus and Gehring (1976a). Because of the high sensitivity of cleavage embryos to X-rays and the time when the irradiations were made, we can be relatively certain that all clones were induced at the blastoderm stage. Their low frequency per fly assures that the overlapping patches are in fact real clones and not caused by two independent induction (all *p* values ≤ 0.02). On the other hand, embryonic damage due to the X-rays is a serious problem for the interpretation of the results, especially for the anterior-posterior overlaps. It is not known to what extent compartment borders would be maintained when a damage in the blastoderm is repaired by regeneration. Schubiger (1971) studied in great detail the regeneration in foreleg discs of mature larvae. The cells capable of regenerating the whole leg seem to lie in the anterior compartment (Fig. 12). The second unknown is the X-ray damage itself in the 3 h embryo and whether this damage would produce regeneration similar to that which occurs in mature discs. This is an important point in interpreting the fact that we found 3 anterior-posterior

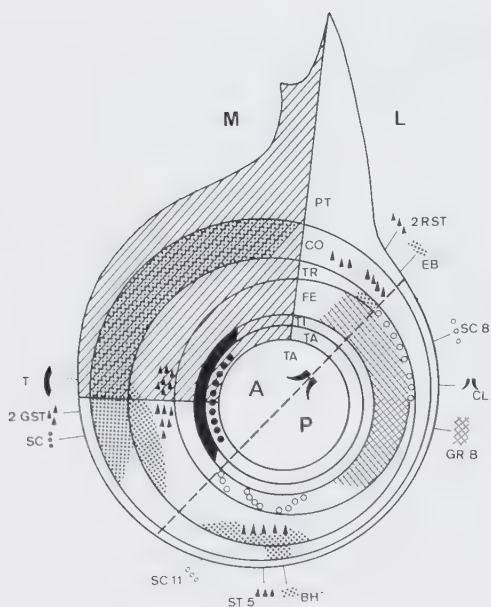


Fig. 12. The anterior-posterior compartment border (broken line) plotted on Schubiger's fate map of the foreleg disc (1968). The upper median quarter (hatched) is capable of regenerating the whole disc (Schubiger, 1971). *M* median, *L* lateral, *A* anterior compartment, *P* posterior compartment, *PT* prothorax, *CO* coxa, *TR* trochanter, *FE* femur, *TI* tibia, *TA* tarsus, *T* transverse bristle rows (tibia), *2 GST* two groups of sensilla trichodea (trochanter), *SC* sex comb, *SC 11* group of 11 sensilla campaniformia (femur), *ST 5* group of 5 sensilla trichodea (trochanter), *BH* isolated bristle in a "hairy island" (coxa), *GR B* large bristles (femur), *CL* claws, *SC 8* group of 8 sensilla campaniformia (trochanter), *EB* edge bristle, *2 RST* two rows of sensilla trichodea (coxa). Dotted areas: anlagen of bristles in coxa and trochanter

overlaps in the first series, but none in the second one. This difference could be due to chance. The probability p for independent events is 0.02 if only the first series is considered. For both series the overlaps are no longer significant ($p=0.12$). The total dose applied (1,000 r) and the clone frequency induced (Table 1) was the same in both series. One could argue that the parameters used in the first series produce X-rays of higher energy and that therefore the damage could be increased and regeneration induced. However, at post-blastoderm stages X-rays with even higher energy did not induce any clones crossing the anterior-posterior compartment borders. Thus, either blastoderms are particularly sensitive to X-rays, or the anterior-posterior compartment border is still unstable at this stage.

Considering the overlaps between dorsal and ventral discs within a segment one could again argue that they are a result of regeneration. In the adults emerging from irradiated blastoderm stages we found no duplications or fusions of the types described by Postlethwait and Schneiderman (1973). Regeneration should be connected with increased clone size, but Wieschaus and Gehring (1976a) using regular mitotic recombination found that the average clone size was identical when half the dose was used. However, this is not a conclusive result since a more extensive study is required to detect damage using a variety of different doses and conditions similar to Schweizer's analysis of the larval genital disc (1972).

The uncertainty about the time of death of the homozygous *Minute* cell, induced simultaneously with the wildtype clone, complicates the problem. The damage set in the blastema by the death of the *M/M* cell immediately after the recombinational event would probably not be sufficient for induction of

regeneration. Regeneration might be induced if the *M/M* cell could divide and form a *Minute* clone condemned to death at some later time.

However, regeneration has never been reported to alter disc-specific determination in larvae from midleg to wing or vice versa. Transdetermination from foreleg to wing is a fairly common event in proliferating cells of imaginal discs (Hadorn, 1966, 1976). Recent studies with midleg discs have not revealed any transdetermination to wing so far (Hadorn and Steiner, unpublished). Thus, if the wing-leg overlaps are a result of regeneration or transdetermination, the blastoderm cells cannot be determined as firmly as larval disc cells are. We consider it much more likely that these overlaps reflect the situation in normal development.

To define the time when the earliest restrictions occur we have to consider the following problems: If we assume that an overlap occurs only if the border is not yet established, then the cell initiating a clone must divide twice between 3 h and the time when determination takes place (once to produce a homozygous *y/y* cell (Fig. 1), and once to produce two marked cells between which a compartment border can fall). Most of the clones induced at 3 h were restricted to a single anterior or posterior compartment of a disc and some overlapped two compartments (dorsal and ventral disc of a segment or anterior and posterior compartment of a disc). One clone extended into three compartments (Fig. 8), and thus the initiating cell must have divided at least three times before both anterior-posterior and wing-leg compartment borders were established. Wieschaus and Gehring (1976a) have shown that *on average*, the precursor cells for imaginal discs divide once between three and ten hours of development. Thus, either there is a great variability in the proliferation of individual cells during embryogenesis, or at least one of these borders must be established later than ten hours. A great variability in clone sizes exists if clones are induced in embryos (Wieschaus and Gehring, 1976a) or in larvae (Becker, 1957; Garcia-Bellido and Merriam, 1971). However, we do not know whether the cell divisions from three to ten hours occur in normal development as well, or if they represent regeneration after irradiation damage.

Even if we consider the anterior-posterior overlaps to be real clones, such overlaps are much rarer than those extending from the dorsal to the ventral disc of a segment. Thus, it is not only likely that the anterior-posterior border exists at or shortly after the blastoderm stage, but that it is established prior to dorsal and ventral disc determination.

It has been suggested that certain compartments are controlled by so-called selector genes (Garcia-Bellido, 1975). If the selector gene is mutant a whole compartment is converted to a different developmental fate. For example the wildtype allele of the mutant *engrailed* is needed for the formation of a normal posterior wing compartment. In a homozygous *engrailed* fly, the posterior wing compartment shows the vein pattern and marginal bristles of an anterior one (Garcia-Bellido and Santamaria, 1972). A functional proof of this conversion was recently reported by Morata and Lawrence (1975). They produced *engrailed* (*en/en*) clones in first instar larvae heterozygous for *M* and *en*. Clones induced in the posterior compartment of the wing frequently crossed the anterior-posterior compartment border. Such overlap was never found for clones induced in

the anterior compartment. These results can be understood if the *engrailed* gene acts only in the posterior compartment. A similar effect of the *engrailed* gene has been reported for the basitarsus of the male foreleg where a secondary sex comb was observed (Tokunaga, 1961). We have found (unpublished) that the original sex comb belongs to the anterior compartment of the foreleg, but that the secondary sex comb of *engrailed* flies is within the posterior compartment.

Garcia-Bellido's model of compartmentalization (1975) requires at least one specific selector gene for each compartment. At the present time only a limited number of mutations can be regarded as possible alleles of selector genes, and more mutations affecting single compartments have yet to be found before the model can be accepted without reservations. It seems feasible that such selector genes would be responsible for the simultaneous establishment of homologous compartments in several discs. Our results demonstrate that such a process of compartmentalization is highly likely to occur in all imaginal discs of the thorax.

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